

Supplementary Table S1. Microbial strains subjected to antimicrobial assays with culture conditions

Strains	Medium	Culture conditions
LAB isolate	MRS	25°C, 48 h
Molds		
<i>Aspergillus flavus</i> ATCC 22546 TM	MEA	30°C, 48 h
<i>Aspergillus fumigatus</i> ATCC 96918 TM	MEA	30°C, 48 h
<i>Aspergillus nidulans</i> PF-3	MEA	30°C, 48 h
<i>Aspergillus ochraceus</i> PF-2	PDA	30°C, 48 h
<i>Penicillium roqueforti</i> ATCC 10110 TM	PDA	25°C, 48 h
Bacteria		
<i>Bacillus cereus</i> ATCC 14579 TM	LB	37°C, 24 h
<i>Escherichia coli</i> O157:H7 ATCC 43895 TM	LB	37°C, 24 h
<i>Listeria monocytogenes</i> ATCC 19113 TM	LB	37°C, 24 h
<i>Micrococcus luteus</i> ATCC 4698 TM	TSB	30°C, 24 h
<i>Pseudomonas aeruginosa</i> KCCM 11328	LB	37°C, 24 h
<i>Salmonella enterica</i> serovar. Typhi ATCC 14028 TM	LB	37°C, 24 h
<i>Staphylococcus aureus</i> KCCM 40881	TSB	37°C, 24 h
<i>Vibrio parahaemolyticus</i> KCCM 11965	NB+2% NaCl	37°C, 24 h

MRS: de Man, Rogosa, and Sharpe; MEA: malt extract agar; PDA: potato dextrose agar; LB: Luria-Bertani; TSB: tryptic soy broth; NB+2% NaCl: nutrient broth containing 2% NB

Supplementary Table S2. Physiological and biochemical properties of LAB isolates

Characteristic	LAB 11	LAB 14	LAB 18	LAB 19	LAB 21	LAB 22
Growth in 6.5% NaCl	+	+	+	+	+	+
Heme-stimulated aerobic growth	-	-	-	-	-	-
Acid production from:						
Glycerol	-	-	-	-	-	-
Erythritol	-	-	-	-	-	-
D-Arabinose	-	-	-	-	-	-
L-Arabinose	+	+	+	+	+	+
Ribose	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+
L-Xylose	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-
Methyl-BD-xylopyranoside	-	-	-	-	-	-
D-Galactose	-	-	-	-	-	-
D-Glucose	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+
L-Sorbose	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-
Inositol	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-
α -Methyl-D-mannoside	-	-	-	-	-	-
α -Methyl-D-Glucoside	+	+	+	+	+	+
N-Acetyl glucosamine	+	+	+	+	+	+
Amygdaline	-	-	-	-	-	-
Arbutin	-	-	-	-	-	-
Esculine	+	+	+	+	+	+
Salicin	w	-	-	-	-	-
Cellobiose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Lactose	-	-	-	-	-	-
Melibiose	-	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+
Inuline	-	-	-	-	-	-
Melezitose	-	-	-	-	-	-
Rafiinose	+	+	+	+	+	+
Starch	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-
β -Gentiobiose	+	+	+	+	+	+
D-Turanose	+	+	+	+	+	+
D-Lyxose	-	-	-	-	-	-
D-Tagatose	-	-	-	-	-	-
D-Fucose	-	-	-	-	-	-
L-Fucose	-	-	-	-	-	-
D-Arabitol	-	-	-	-	-	-
L-Arabitol	-	-	-	-	-	-
Gluconate	-	-	+	+	+	+
2-Keto-gluconate	+	+	+	+	+	+
5-Keto-gluconate	+	+	+	+	+	+

The API 50 CHL system was used for carbohydrate assimilation by LAB; w, weakly positive; +, positive; -, negative.

Supplementary Table S3. Enzymatic activities of *L. aenigmaticum* LS4 as determined using the API ZYM kit

Unit: nmol	
Enzyme	Activity of <i>L. aenigmaticum</i> LS4
Alkaline phosphate	0
Esterase (C4)	0
Esterase lipase (C8)	0
Lipase (C14)	0
Leucine arylamidase	10
Valine arylamidase	0
Cystine arylamidase	0
Trypsin	0
α -Chymotrypsin	0
Acid phosphatase	0
Naphthol-AS-BI-phosphohydrolase	5
α -Galactosidase	10
β -Galactosidase	≥ 40
β -Glucuronidase	0
α -Glucosidase	20
β -Glucosidase	0
N-Acetyl- β -glucosaminidase	0
α -Mannosidase	0
α -Fucosidase	0

Enzymatic activities indicate nanomoles of hydrolyzed substrate after 4 h of incubation at 25 °C; 0 = no activity, 5 = 5 nmol, and 10 = 10 nmol.

According to the manufacturer's instructions, \geq or $<$ 20 nmol of substrate hydrolyzed were defined as positive and negative reactions, respectively.

Supplementary Table S4. Functional annotations of the coding sequences in *L. aenigmaticum* LS4 and their deduced proteins



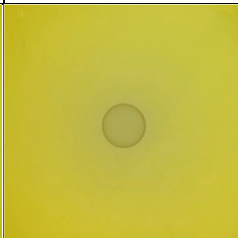

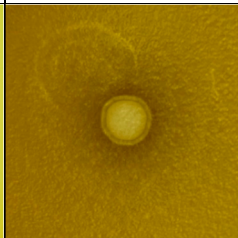
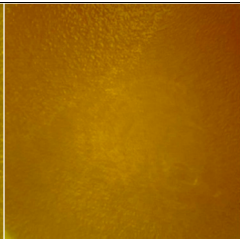
Description		Strain LS4	Annotation results				
		CDS length for LS4 (a.a)	CDS length (a.a)	Query coverage (%)	Identity (%)	Predicted protein *	
Cold-shock response related	Cold shock protein (CSP)	66	66	100.00	100.00	<i>cspA</i> ; cold shock protein	
		74	74	100.00	95.95	<i>cspA</i> ; cold shock protein	
	DEAD-box RNA helicase	542	538	100.00	98.34	<i>deaD</i> , <i>cshA</i> ; ATP-dependent RNA helicase DeaD [EC:3.6.4.13]	
		454	454	100.00	99.56	<i>cshB</i> ; ATP-dependent RNA helicase CshB [EC:3.6.4.13]	
	Ribonuclease (RNase)	116	116	100.00	100.00	<i>rnpA</i> ; ribonuclease P protein component [EC:3.1.26.5]	
		518	518	100.00	98.46	<i>rny</i> ; ribonuclease Y [EC:3.1.-.-]	
		774	774	100.00	98.32	<i>rnr</i> , <i>vacB</i> ; ribonuclease R [EC:3.1.13.1]	
		328	328	94.00	91.26	<i>rnhC</i> ; ribonuclease HIII [EC:3.1.26.4]	
		600	600	100.00	98.33	<i>rnj</i> ; ribonuclease J [EC:3.1.-.-]	
		148	148	100.00	100.00	<i>rnhA</i> , RNASEH1; ribonuclease HI [EC:3.1.26.4]	
		254	254	100.00	93.31	<i>rnhB</i> ; ribonuclease HII [EC:3.1.26.4]	
		124	124	100.00	94.35	<i>rnhA</i> , RNASEH1; ribonuclease HI [EC:3.1.26.4]	
		314	314	100.00	97.45	<i>rnz</i> ; ribonuclease Z [EC:3.1.26.11]	
		233	233	100.00	99.14	<i>rnc</i> , DROSHA, RNT1; ribonuclease III [EC:3.1.26.3]	
		186	186	100.00	94.09	<i>rnmV</i> ; ribonuclease M5 [EC:3.1.26.8]	
		560	560	100.00	99.64	<i>rnj</i> ; ribonuclease J [EC:3.1.-.-]	
		ABC and efflux MFS transporter	301	301	100.00	98.01	ABC-2.A; ABC-2 type transport system ATP-binding protein
	Ribosomal protein	49	49	100.00	100.00	RP-L33, MRPL33, rpmG; large subunit ribosomal protein L33	
		49	49	100.00	100.00	RP-L33, MRPL33, rpmG; large subunit ribosomal protein L33	
	rRNA/tRNA modification	253	253	100.00	96.44	tRNA ^I Val (adenine37-N6)-methyltransferase [EC:2.1.1.223]	
		295	295	100.00	96.61	<i>ksgA</i> ; 16S rRNA (adenine1518-N6/adenine1519-N6)-dimethyltransferase [EC:2.1.1.182]	
		266	255	95.00	96.08	<i>truA</i> , PUS1; tRNA pseudouridine38-40 synthase [EC:5.4.99.12]	
		302	302	100.00	95.03	<i>truB</i> , PUS4, TRUB1; tRNA pseudouridine55 synthase [EC:5.4.99.25]	
	Safety related	Hemolysis	212	212	100.00	99.06	<i>hlyIII</i> ; hemolysin III
		Vancomycin	377	377	100.00	96.82	<i>ddl</i> ; D-alanine-D-alanine ligase [EC:6.3.2.4]
Functional related	Sucrase	1528	1526	100.00	93.46	Dextranucrase [EC:2.4.1.5]	
		1443	1442	100.00	95.08	Dextranucrase	
		1162	1228	100.00	89.26	Levansucrase / Inulosucrase	
	Bacteriocin	71	60	91.00	38.46	Penocin A	
		48	55	93.00	50.98	Enterocin X chain beta	

* Functional annotations of CDSs for cold shock response and safety related genes were performed using KEGG BlastKOALA. CDSs for sucrase and bacteriocin were annotated using KEGG BlastKOALA and BAGEL 4, respectively.

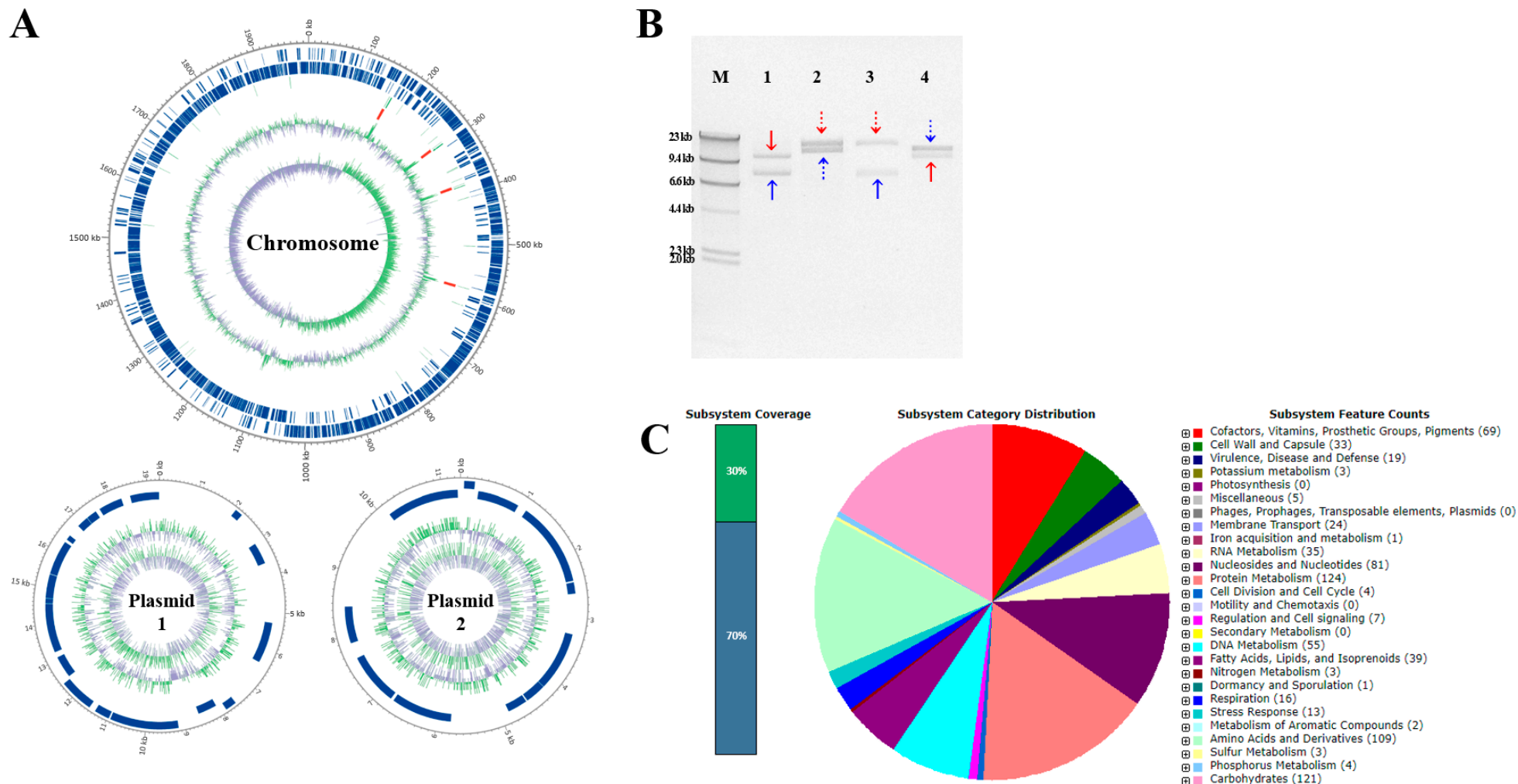
Supplementary Table S5. Amino acid sequence alignments of pediocin-like bacteriocins (IIa), two-peptide bacteriocin (IIb) and the predicted bacteriocins from *L. aenigmaticum* LS4.

Class	Bacteriocin	Leader sequence		Mature sequence	
		Homology (%) [*]	Sequence	Sequence	Homology (%)
IIa	Pediocin PA	16.67	-----MKKIEKLTEKEMANIIGG	YY GNGV XC KXXCXVDWG A	18.18
	Sakacin A	22.22	-----MNNVKELSMTELQTIITGG	-KY Y--GNGV--TCGKHSCSVDWGKATTCI INNGAMAWATGGHQGNH KC	21.95
	Leucocin A	33.33	-----MNNVKELSMTELQTIITGG	-KY Y--GNGV--HCTKSGCSVNWGEAFSAGVHRLANG--GNGFW-----	18.92
	Penocin A	27.78	-----MNNVKELSMTELQTIITGG	-KY Y--GNGV--HCGKKT CYVDWQATASIGKI IVNGWTQHGPWAHR--	38.10
	LS4-1	-	---MKSLQDFQTMNHTQLAQVNGG	KRVYIPNGGAWLDSNTGKGGVDWNVAVPALGSI MVNGWAQNGPLAHLHP	-
IIb	Enterocin X _β	60.00	MKKYNELSKKELLQIQGG	GXXXG motifs	
	LS4-2	-	---MNKLSQEELSQISGG	IAPII VAGLGYLVKDAWDHSDQII SGFKKGWNGGRRK--	33.33
				IWPLVVAG--YLG YQAFEHSDQIVAGW----NHAGKKHL	-

^{*} Predicted bacteriocins in *L. aenigmaticum* LS4 were compared with other bacteriocins using CLUSTAL W (<https://www.genome.jp/tools-bin/clustalw>). Bold red characters indicated conserved sequences.

A		B		C	
<i>Lactobacillus</i> sp. ATCC 33222™	<i>L. aenigmaticum</i> LS4	<i>Lactobacillus</i> sp. ATCC 33222™	<i>L. aenigmaticum</i> LS4	<i>Enterococcus faecalis</i> ATCC 29212™	<i>L. aenigmaticum</i> LS4
					

Supplementary Figure S1. Biogenic amine production by *L. aenigmaticum* LS4 determined using the screening plate method. Bover-Cid and Holzapfel medium supplemented with 1% ornithine (A), 1% histidine (B), or 1% tyrosine (C). *Lactobacillus* sp. ATCC 33222™ and *Enterococcus faecalis* ATCC 29212™ were used as positive controls.



Supplementary Figure S2. Genomic features of *L. aenigmaticum* LS4. (A) Circular representation of the chromosome and the two plasmids genomes of *L. aenigmaticum* LS4. From the outside circle to the center; CDS on forward strand, CDS on reverse strand, tRNA, rRNA, GC content and GC skew. **(B)** Agarose gel electrophoresis of plasmids 1 and 2. M, size marker; 1, ccc plasmids 1 and 2; 2, linear plasmids 1 and 2 digested with *Age* I, 3; linear plasmid 1 digested with *Sph* I, 4; linear plasmid 2 digested with *Nco* I (→ : ccc plasmid 1, → : ccc plasmid 2, → : linear plasmid 1, → : linear plasmid 2). **(C)** Subsystem feature of the genomic sequence of *L. aenigmaticum* LS4 analyzed using the RAST server. For the 1983 coding sequences predicted by the RAST server, the subsystem coverage was 30% (the green bar), which contributed to a total of 207 subsystems. The blue bar refers to the percentage of proteins not included in the subsystems.